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Effect of Sapodilla (*Manilkara zapota* L.) Leaves Extract on the Lipid Levels of Diabetic Mice Induced with Alloxan

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Abstract

Sapodilla leaves extract has hypocholesterolemic and high antioxidant activity with potential to protect the body from ROS effect. This study used 25 male mice which were divided into 5 groups. The groups consisted of diabetic mice control, diabetic mice treated with pioglitazone 20mg/kg, normal mice control, diabetic mice treated with sapodilla leaves extract at 70mg/kg and 140mg/kg. The results of the study showed that 14 days administration of sapodilla leaves extract at 70 mg/kg and 140 mg/kg decreased the TG, LDL, and TC, while at 140 mg/kg, sapodilla leaf extract increased the HDL level.

Key words: Alloxan, Diabetes, Lipid, Sapodilla.

Diabetes mellitus is a metabolic disorder common in humans and animals. The disorder is marked by increase in blood glucose level that can be caused by abnormalities in insulin secretion, insulin action, or both. Insulin plays critical role in regulating lipid metabolism as it inhibits the hormone sensitive lipase (HSL) in adipose tissues that leads to decrease in secretion of free fatty acids (Solikhah, 2020). Insulin resistance or its deficiency can lead to the activation of HSL that will cause an increase of free fatty excretion from adipose tissue (Solikhah, 2021). Dyslipidemia in diabetic patients are characterized by increase in triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), and decrease in high density lipoproteins (HDL).

Other than generic drugs, people have also resorted to alternative medicines, such as herbal medicines (Khairullah *et al*., 2020, 2021). Flavonoid, a phytochemical found in the sapodilla leaves extract has strong antioxidant activity that supresses hyperglycemia in diabetic patients (Solikhah *et al*., 2023). Seed, leaves, and root extracf of *Manilkara zapota* L. contains phytochemicals such as saponins and flavonoids with strong antioxidant effect (Islam *et al*., 2021). Antioxidants from plants are able to minimize the severity of diabetes mellitus complications caused by excess free radicals from glucose oxidation (Karle *et al*., 2022). Based on a study by Karle *et al*. (2022) Sapodilla fruit peel extract at 300 mg/kg body weight resulted in decrease of TG and LDL while increased the HDL level of blood serum in alloxan induced diabetic rats.

Materials and Methods

Healthy 3 months old male mice (Mus musculus) with body weight of 25-35 g were used as animal models. Sapodilla leaves (*Manilkara zapota* L.) taken from Puri District, Mojokerto Regency, East Java Province, samples of Manila sapodilla leaves (*Manilkara zapota* L.) were selected purposively without comparing with

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similar plants from other areas, ethanol 96%, water, Hi-Pro-Vite Pokphand 511 feed, sawdust, NaCl 0,9%, aquades, alcohol 70%, alloxan, pioglitazone, ketamin, xylazine, and CMC Na 0.5%. Tools used in this study were glucometer (Accu-Check Instant), blood glucose test strip, serum lipid diagnostic kit (Azmoon Kit), and automatic analyzer abbot (Alcyon 300), centrifuge, vacutainer, oven, strainer, rotary evaporator, Beaker glass, stirring rod, aluminium foil, surgical scissor, scalpel and blade, 1 mL syringe, oral gavage, plastic cage, digital scale, fridge, coolbox, water bottle, and feed container.

This study can be classified as posttest only control group design. Ethical clearance number 2.KEH.068.06.2022 is obtained from faculty of veterinary medicine Universitas Airlangga. Based on Dahlan's (2011) formula, the minimum expected sample was 5. The number of experimental animals used in this study was 25 healthy male mice (*Mus musculus*) with 5 mice in each treatment group.K(-) is the diabetic control group, K(+) is diabetic mice given pioglitazone 20mg/kg BW, P0 is a normal mice control, P1 is diabetic mice given sapodilla leaves extract at 70 mg/kg BW, and P2 is diabetic mice given sapodilla leaves extract at 140 mg/kg BW. Pioglitazone and sapodilla leaves extract were given once orally every single day for 14 days. Mice then were terminated to collect blood sample via cardiac puncture to analyze its lipid serum level using diagnostic kit and automatic analyzer. Numerical data of TC, TG, HDL, and LDL levels were analyzed with Statistical Product and Service Solution program.

Results and Discussion

Normal TG level of male mice was found to be 122.4 ± 9.34 mg/dL (Kurniandari *et al*., 2017). TG levels following administration of sapodilla leaves extract at 70 mg/kg BW and 140 mg/kg BW groups were found to be the lowest compared to other groups. Oleic acid can affect lipid profile as it decreases postprandial lipoprotein oxidation and lowers TG level (Granado-Casas and Mauricio, 2019). Phenol compounds are able to protect the body from non communicable disease as it has antioxidant activity and able to regulate cellular function such as enzyme inhibition, modify gene expression, and protein phosphorylation (Muhammad Abdul Kadar *et al*., 2021). A research by Al-Abbasi and Kazmi (2022) indicates that myricetin, a compound that can also be found in sapodilla leaves extract has the ability to lower TG.Myricetin will activate glucagon-like peptide 1 receptor (GLP-1R) that stimulates insulin secretion. Insulin has a crucial role in regulating lipid metabolism, especially in lowering production and increasing catabolism of VLDL through activating LPL resulting in decrease of TG (Verges, 2020).

Increase in HDL is suspected to be the result of an increase of LPL/Hepatic lipase ratio on DMT1 patient with good glycemic

Table I. Description of Mice Blood Lipid Serum Average

Groups	MEAN±SD (mg/dL)			
	TG	HDL	LDL	TC
Normal Control	183.20 ± 4.08^{a}	58.20 ± 2.04^{bc}	56.00 ± 2.64^{b}	151.00 ± 2.34^{a}
Diabetic Control	238.40 ± 7.54^{b}	61.80 ± 2.68^{c}	101.60 ± 5.07^{d}	211.00 ± 8.57^{b}
Pioglitazone 20 mg/kg BW	278.00 ± 8.00^{c}	50.00 ± 5.00^{a}	42.80 ± 2.05^{a}	148.40 ± 3.21^{ac}
Sapodilla leaves extract 70 mg/kg BW	125.60 ± 5.36^{d}	54.00 ± 4.18^{ab}	64.60 ± 8.82^{c}	143.80 ± 3.83^{c}
Sapodilla leaves extract 140 mg/kg BB	91.20 ± 4.15^{e}	72.00 ± 6.00^{d}	55.00± 5.00^{b}	145.20 ± 5.35^{c}

^{a,b,c,d,e} same superscript shows there are no significant difference in Duncan or Mann-Whitney test

control, meanwhile on patient with poor glycemic control, there is an increase of methionine specific residue from apoA1 oxidation that lowers HDL atheroprotective function (Verges, 2020). Oxidative stress may change HDL practicles to oxidized HDL that last longer in blood circulation, in the end contributing to atherosclerosis incident (Solikhah *et al*., 2023). Normal HDL level of mice were found to be 64 ± 5.70 mg/dL (Kurniandari *et al*., 2017). Statistical analysis shows HDL level of normal control group did not differ significantly with diabetic control or sapodilla leaves extract at 70 mg/kg BW group. Lowest mean HDL level are found in pioglitazone 20 mg/kg BW group, However statistically it does not differ significantly if compared to sapodilla leaves extract 70 mg/kg BW. Highest mean HDL level can be found in sapodilla leaves extract 140 mg/kg BW group.

Sapodilla leaves extract contains linoleic acid, oleic acid, apigenin, phenolic compound, myricetin, and caffeic acid (Maseera *et al*., 2022). Linoleic acid, oleic acid, and caffeic acid have the benefit of increasing HDL (Granado-Casas and Mauricio, 2019). Research by Al-Abbasi and Kazmi (2022) and Zhao *et al*. (2022) shows kaempferol and myricetin is able to increase glutathione (GSH), antioxidant activity, regulating lipid metabolism, and increasing HDL level, oxidative stress results in increased tissue lipid peroxidation leading to overproduction of free radicals and inactivation of membrane-bound antioxidant enzymes. Apigenin can lower the production of hepatic glucose by decreasing hepatic lipid peroxidation level through an increase of antioxidant activity of superoxide dismutase (SOD) enzyme, catalase (CAT), and GSH in mice liver (Dinda *et al*., 2020).

Based on research (Kurniandari *et al*., 2017), mean LDL level of male mice is 134.6 ± 16.57 mg/dL. Diabetic control group has the highest mean LDL level. Disruption in lipid metabolism in diabetes mellitus can result in increase of plasma LDL levels (Zhao *et al*., 2022). Pioglitazone 20 mg/kg BW, sapodilla leaves extract 70 mg/kg BW and 140 mg/kg BW treated groups showed a significant decrease in the LDL levels compared to diabetic control group. LDL level on administration of sapodilla leaves extract 140 mg/kg BW shows almost the same value as the normal control group.

Oleic acid and linoleic acid have the benefit of lowering LDL levels (Granado-Casas and Mauricio, 2019). Myricetin is able to lower LDL level inmice serum (Zhao *et al*., 2022). Apigenin is found to have a role in protecting the body from cardiometabolic disease that can be caused by metabolic dysfunction such as dyslipidemia (Xu *et al*., 2022). Apigenin is able to encourage absorption of hepatic LDL and its free radical scavenging activity are able to lower LDL oxidation and decrease its transport to tissue (Barky *et al*., 2020).

Mean normal TC level of male mice is found to be 186.6 ± 10.71 mg/dL (Kurniandari *et al*., 2017). Diabetic control mice has the highest mean TC level and significantly differ statistically in comparison with other groups. Sapodilla leaves extract 70 mg/kg BW and 140 mg/kg BW treated mice showed a decrease in TC levels in contrast with diabetic control mice, but there is no significant difference in TC value between dosages.

Research by Al-Abbasi and Kazmi (2022) shows that myricetin is able to lower cholesterol level. Linoleic acid is proven to lower plasma cholesterol levels. Apigenin can lower free fatty acid, plasma apoB, and cholesterol by promoting hepatic LDL-c absorption (Barky *et al*., 2020). Apigenin induce activation of adenosine monophosphate-activated protein kinase (AMPK) that lower sterol regulatory element-binding protein (SREBP-1 and SREBP-2) level, causing decrease in the synthesis of cholesterol, fatty acid, and TG in liver (Xu *et al*., 2022). Research by Dinda *et al*. (2020) indicate that apigenin is able to lower TC and TG, allegedly because apigenin helps to lower HMG-CoA reductase and acetylCoA cholesterol-oacetyl transferase (ACAT) function.

Conclusion

Based on the results of this study, 14 days administration of sapodilla leaves extract 70 mg/kg and 140 mg/kg showed a decrease in the groups TG, LDL, and TC groups while sapodilla leaves extract 140 mg/kg showed increase in HDL levels.

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